Pheromonal Behavior in the Marine Snail *Littorina littorea* Linnaeus

BY

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INTRODUCTION

Pheromones are chemicals secreted into the environment that provide an intra-specific communication system. They have been identified in most of the principal animal phyla but the large majority of scientific papers are on chordates and arthropods (Wilson, 1970). Two reports on pheromones in marine snails have come to my attention (Snyder, 1967; Snyder & Snyder, 1971). The author previously wrote on evidence for a pheromone in *Littorina littorea* Linnaeus, 1758 (Dinter & Manos, 1972). The present study elaborates on the last paper and demonstrates that males produce a substance (or substances) during the mating season which attracts both males and females. The results are inconclusive with regard to females.

The experiments were designed to test for the presence of a pheromone by allowing a snail to enter one of the two test tubes, one contained untreated sea water and the other contained test water in which another animal had stayed for four hours. Each responding snail permitted to make the run was also tested once to see which of the two tubes it preferred when both were filled with untreated sea water (control).

MATERIALS and METHODS

Littorina littorea were collected once or twice a week at the Northeastern Marine Science Institute at Nahant, Massachusetts. Only sexually mature snails were used (Williams, 1970).

The experiments were performed from February 15, 1972 to April 27, 1972. On March 21 a plankton tow near the Marine Science Institute revealed *Littorina littorea* egg capsules. From this time on the sea aquaria were checked for egg capsules after each collection of fresh snails. They were found until the end of April. It is cited in the literature that *Littorina littorea* has one mating season a year although the onset and end of it vary considerably (Linke, 1933; Williams, 1964). It is likely that most of this work was conducted during the mating season.

Immediately after collection the animals were separated according to sex. Three-gallon capacity containers were used for the experiments. Two glass test tubes (26 × 200 mm) were attached to a glass plate with waterproof electrical tape. A plastic platform taped 50 mm below the test tube openings served as a starting holder for the experimental animal (Dinter & Manos, 1972). A snail was inserted into the right tube; both tubes were then filled with sea water, stoppered, and set aside for four hours to allow the pheromone, if present, to concentrate. A snail was only used once during an experiment. After this time the snail was quickly removed with a spatula and the tube was restoppered. This set-up was submerged vertically, test tube openings down, into a sea water filled container and the stoppers removed. A test snail was placed onto the platform and the container was covered with a lighttight box. After 10 minutes the aquarium was uncovered and the position of the snail recorded. It is important to note that for each snail run a fresh set-up of glass plate, test tubes, and control and test sea water was used. (This is in contrast to the previously reported experiments in which the set-up was changed only if a snail had entered the control tube. The fact that both males and females were allowed to enter the experimental tube during those experiments would not have allowed detection of different behavior for males and females). This way there was no possibility for previously run animals to leave a scent or trail on the tubes or plate.

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The experiments were run in the dark for two reasons: to eliminate phototactic movements and to take advantage of the fact that periwinkles are negatively geotropic in the dark. Under these conditions the snails show a tendency to move vertically and to the left (DINTER & MANOS, 1972), consequently the control tube (sea water) is located on the left side and the experimental tube on the right side. A control run was performed on each snail under the same conditions except that both tubes were filled with untreated sea water. All data presented in this report are for snails which entered one tube or the other. At the beginning of an experiment the water temperature was 9° C and at the end 16±2° C. Tests were usually carried out during the daytime hours.

EXPERIMENTS AND RESULTS

Experiment I was set up to see if males produce a substance that would attract females during this season. One male was kept in the right tube for four hours. The female was placed on the platform to choose between normal and "male" water. Fifty-two females were tested. The data show attraction of females by "male water."

Experiment II was performed to test whether or not males atract each other by chemical means during the mating season. A total of 80 males were tested. The results show that "male" water does attract males. This is demonstrated by the use of the null hypothesis which is checked by means of the chi-square test. If the data show a significant statistical difference between control and experimental runs the null hypothesis can be rejected. In this case it could be rejected. (Table 1).

Table 1

Preferential entry of left or right tube

Experiment No.	Run	No. of snails entering left tube	right tube	X^2	p
I male water	control	21	3	13.2	<0.005
female run	test	9	18		
II male water	control	24	12	5.6	<0.025
male run	test	17	28		
III female water	control	19	22	0.08	>0.75
female run	test	17	18		
IV female water	control	11	9	0.001	>0.975
male run	test	13	15		
V male water from tank	control	14	4	6.3	<0.025
female run	test	13	22		

Whether females are attracted by " female " water was tested in experiment III. Eighty females were run. Due to the anomalous behavior of the control animals, the results are inconclusive.

Experiment IV was set up to see if " female " water attracted males. Fifty-two males were tested. Again the control runs were not as expected.

Since each snail had been left in the test tube for four hours it also secreted a mucus trail on the glass. It was of interest, then, to see whether or not the "male" water alone was attractive. Freshly collected animals, sorted out by sex, were kept separately in approximately 3 liter-capacity seawater aquaria for 2 days. The right tube of the set-up for this experiment was filled with " male " water taken from the aquarium. Experiment V showed that this water without glassbound mucus attracted females.

DISCUSSION

In experiments I & II females and males respectively were attracted to "male" water. It may be hypothesized that the male of the species secretes a pheromone that attracts males as well as females during the reproductive season.

No statement can be made about the efficacy of female water to attract snails since the control runs did not show the usual pattern of predominantly left moving behavior.

A closer examination of the data for experiments III & IV reveals that there is no difference between control and testwater run. Females entered the right tube in both cases in almost equal numbers (22 control, 18 experimental). Neither was there a significant difference of females entering the left tube. Males coming in contact with female water also behaved similarly in control water. The same tests must be repeated in the next mating season to determine to what extent "female water" does or does not attract either males or females.

Experiment V shows that "male water" alone (without mucus trail on the glass tube), attracts female periwinkles. It is still possible that some component of the mucosa is soluble in water and is responsible for the attraction of snails.

What role the substance(s) may play in sexual or aggregational behavior of the marine periwinkle remains to be determined.

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